# 1. 'A' Allele, CYP2D6'3, A21337 deletion, Frameshift resulting in zero enzyme activity

OCT 2 5 2004

### Appln. No. 09/880,732 Amendment dated October 25, 2004 Response to Drawing Requirement of August 25, 2004

CYPmut(+)B1930, 17mer,65%GC, Tm=54C

B1930 (A) 30-3'NH2

CYPwt (-)

CYPmut (+) B1930 (A) 30-3'NH2

Wild Type(+)

CCCTTACCCGCATCTCCCCACCCCCAGGACGCCCCTTTCGCCCCAACGGTCT CCCTTACCCGCATCTCCCACCCCCAAGACGCCCCTTTCGCCCCAACGGTCT

51.

SEQ ID NO:26 SEQ ID NO:27

CCCAAGACGCCCTTTC(A)30 -3' NH2

5'- CCCAAGACGCCCTTTC 5'- CCCAAGACGCCCCTTTC(

NH2 3'- GGGTCCTGCGGGGAAG 3'- (A) 30GGGTCCTGCGGGGAAAG

ID NO:22 ID NO:23

ID NO:24 ID NO:25

SEQ SEQ SEQ SEQ

Mut (+)

Tm=56C

CYPwt(-) B1930,17mer,71%GC,

SEQ ID NO:6	5'- GCTAACTGAGCACAGGATGACC -3' NH2	CYPwt(+)A2624, 22mer,54%GC, Tm=63-64C	į
SEQ ID NO:7	5'- GCTAACTGAGCACAGGATGACC(A)30 -3' NH2	CYPwt (+) A2624 (A) 30-3'NH2	
SEQ ID NO:8	5'- CTAACTGAGCACAGGATGACC(A)30 -3' NH2	CYPwt (+) A2625 (A) 30-3'NH2	
SEQ ID NO:9	5'- CTAACTGAGCACAGGATGAC(A)30 -3' NH2	CYPwt (+) A2625b (A) 30-3'NH2	
SEQ ID NO:10	5'- GCTAACTGAGCAC-GGATGACC -3'NH2	CYPmut (+) A2624,21 mer,57%GC, Tm=61-63C	
SEQ ID NO:11	5'- GCTAACTGAGCAC-GGATGACC(A)30 -3' NH2	CYPmut (+) A2624 (A) 30-3'NH2	
SEQ ID NO:12	5'- CTAACTGAGCAC-GGATGACC(A)30 -3' NH2	CYPmut (+) A2625 (A) 30-3'NH2	
SEQ ID NO:13	5'- CTAACTGAGCAC-GGATGAC(A)30 -3' NH2	CYPmut(+)A2625b(A)30-3'NH2	
SEQ ID NO:14 5	-2612 - GCTGGATGAGCTGCTAACTGAGCACAGGATGACCTGGGACCCAGCCCAGCC -3'	Wild Type(+)	Resp
ID NO:15	5'- GCTGGATGAGCTGCTAACTGAGCAC-GGATGACCTGGGACCCAGCCCAG	Mut (+)	Onse
			. 10
2. 'B' Allele,	'B' Allele, CYP2D6*4, G1934A, Spliceing defect resulting in zero enzyme activity		I
A.	A. wt Probe - CYPwt(-)B1922 (C/A to mut at base 5) & CYPmut(+)B1922 (A/C to mut at base 13)		Зер
	1934		l:
SEQ ID NO:16	NH2 3'- GAGGGTGGGGGTCCTGC -5'	CYPwt(-)B1922, 17mer,76%GC, Tm=66C	ace
SEQ ID NO:17	5' - CTCCCACCCCAGGACG -3'NH2	CYPwt (+) B1922-Target	em
SEQ ID NO:18	5'- CTCCCACCCCAAGACG -3' NH2	CYPmut (+) B1922, 17mer, 71%GC, Tm=58-60C	en
SEQ ID NO:19	NH2 3'- GAGGGGGGTTCTGC -5'	CYPmut(-)B1922- Target	t S
	-1909		She
SEQ ID NO:20 5	· - CCCTTACCCGCATCTCCCACCCCCAGGACGCCCCTTTCGCCCCCAACGGTCT -3'	WildType(+)	et
SEQ ID NO:21 5	'- CCCTTACCCGCATCTCCCACCCCCAAGACGCCCCTTTCGCCCCCAACGGTCT -3'	Mut (+)	иди
œ œ	CYPwt(-)B1930 (C/A to mut at base 13) and CYPmut(+)B1930 (A/C to wt at base	./C to wt at base 5)	,

# FIGURE 6B

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SEQ

SEQ

SEQ SEQ SEQ SEQ SEQ SEQ

SEQ

Allele, CYP2D6\*9, G2702-A2704 deletion, decreased enzyme activity

### Pred Tm=57 CYPmut (+) E3009, 19mer, 58%GC, Pred Tm=59C Tm=60C Tm=60C CYPmut(+)C2691, 21mer, 57%GC, CYPwt(-)E3009, 19mer,53%GC, 22mer, 55%GC, A. wt Probe - CYPwt(-)E3009 (T/C to mut at base 5) & CYPmut(+)E3009 (C/A to wt at base 15) (A) 30-3'NH2 CYPmut (+) C2692 (A) 30-3'NH2 CYPmut (+) E3009 (A) 30-3'NH2 CYPwt (+) C2691 (A) 30-3'NH2 CYPwt (+) C2692 (A) 30-3'NH2 CYPwt(-) E3009(A)30-3'NH2 Wild Type (+) CYPmut (+) C2691 CYPwt (+) C2691, Allele, CYP2D6\*7, A3023C, H324P amino acid change results in zero enzyme activity Wild Type(-) Mut (-) TGGGGCCTCCTGCTCATGATCCTACCTCCGGATGTGCAGC | GTGAGCCCATC -3' 3' - TGACTCCGGAAGGACCGTCTCTACCT - - CCACTCTCACCGACGGTGCCAC -5' -5, TGGGGCCTCCTGCTCATGATCCTACATCCGGATGTGCAGC | GTGAGCCCATC 5'- GCAGAGATGGA---GGTGAGAGTG(A)30 -3' NH2 5'- CAGAGATGGA---GGTGAGAGTG(A)30 -3' NH2 3' - TGACTCCGGAAGGACCGTCTCTACCTCTTCCACTCTCACCGACGGTGCCAC 5'- GCAGAGATGGAGAAGGTGAGAG -3'NH2 5'- GCAGAGATGGAGAAGGTGAGAG(A)30 -3' NH2 5'- CAGAGATGGAGAAGGTGAGAG(A)30 -3' NH2 5'- GCAGAGATGGA---GGTGAGAGTG -3' NH2 5'- GCTCATGATCCTACCTCCG(A)30 -3'NH2 5' - GCTCATGATCCTACCTCCG -3'NH2 3'- (A) 30CGAGTACTAGGATGTAGGC -5' NH2 3' - CGAGTACTAGGATGTAGGC -5 -2998 NH2 2, -2, -NO:40 NO:29 NO:30 NO:34 NO:37 NO:38 ID NO:35 NO:41 NO:32 NO:28 NO:31 NO:36 NO:39 NO:33 П SEQ ID Ω -団 П

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# CYPmut (+) E3018, 19mer, 63%GC, Tm=62C CYPwt(-)E3018,19mer,58%GC,Tm=60 CYPmut(-) E3018- Target CYPwt(+)E3018- Target Wild Type(+) Mut (+) TGGGGCCTCCTGCTCATGATCCTACCTCCGGATGTGCAGC | GTGAGCCCCATC -3' TGGGGCCTCCTGCTCATGATCCTACATCCGGATGTGCAGC | GTGAGCCCATC -3' NH2 5, 5' - CCTACCTCCGGATGTGCAG 3'- GGATGTAGGCCTACACGTC 5' - CCTACATCCGGATGTGCAG 3'- GGATGGAGGCCTACACGTC NH2 -2998 2, -2, -

-3038-Intron Start

NO:47

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NO:46

SEQ

ID NO:43 NO:44 NO:45

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SEQ

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SEQ

to wt at base 6)

CYPwt(-)E3018 (T/C to mut at base 14) and CYPmut(+)E3018 (C/T

-3038-Intron Start

# Appln. No. 09/880,732

# Amendment dated October 25, 2004

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		Replacement	Shee

CYPwt(+)G1840(A)30-3'NH2,18mer,67%GC, Tm=60 CYPmut (+) G1840 (A) 30-3'NH2,18mer, 61%GC, CYPmut(+)T1785, 17mer,71 %GC, CYPwt (+) T1785, 18mer, 67%GC, CYPmut (+) T1785 (A) 30-3'NH2 CYPmut (+) T1786 (A) 30-3'NH2 (A) 30-3'NH2 CYPwt (+) T1786 (A) 30-3' NH2 CYPwt(-) G1840(A)30-3'NH2 CYPwt (+) T1785 T1795 deletion, Frameshift resulting in zero enzyme activity Wild Type (+) GTGCCGCCTTCGCCACTCC | GGTGGGTGATGGGCAGAAGGGCACAAAGCGGG -3' 5'- GTGCCGCCTTCGCCACTCC TGTGGGTGATGGGCAGAAGGGCACAAAGCGGG -3' 5'-GGGCAAGAAGTCGCTGGAGCAG-GGGTGACCGAGGAGGCCGCCTGCCT -3' 5' - CACTCCTGTGGGTGATGG(A)30 -3' NH2 5' - GCTGGAGCAG-GGGTGAC(A)30 -3' NH2 5' - CTGGAGCAG-GGGTGAC(A)30 -3' NH2 CTGGAGCAGTGGGTGAC(A)30 -3' 5' - GCTGGAGCAGTGGGTGAC(A)30 -3' 5' - GCTGGAGCAG-GGGTGAC -3' NH2 5' - GCTGGAGCAGTGGGTGAC -3' NH2 5' - CACTCCGGTGGGTGATGG (A) 30 NH2 3'- (A) 30GTGAGGCCACCACTACC Exon 3 end- |-1846 CYP2D6\*6, -1773 5, -Allele, NO:49 NO:51 NO:52 NO:55 SEQ ID NO:59 SEQ ID NO:60 NO:54 NO:57 NO:50 NO:53 NO:56 NO:58 U CI H H П I SEQ ID SEQ SEQ SEQ SEQ SEQ SEQ

G1846T, Stop codon, zero enzyme activity

CYP2D6\*8,

Allele,

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FIGURE 6C

designs was to find region somewhere between the PCR primers were it would be easy to discriminate between 2D6 and 7. 2D6/2D7/2D8 Controls - The 2D6/7/8 probes were designed in the 1600 region of the 2D6 gene. The purpose of the its two pseudogenes, 2D7 and 2D8. The purpose of the designs is to demonstrate that the PCR amplicon is from the gene, not one of the pseudogenes.

SEQ ID NO:61		5' - GACCAGGGGGAGC-ATAGG(A)30-3' NH2	CYP2D6wt (+) 1607 (A) 30-3'NH2
SEQ ID NO:62		5' - GACCTTGTGGAGCGCCAG(A)30-3' NH2	CYP2D7wt(+)1607(A)30-3'NH2
SEQ ID NO:63		5' - GACCAGGAAAAGC-ACAGG(A)30-3' NH2	CYP2D8wt (+) 1607 (A) 30-3'NH2
SEQ ID NO:64		5' - GACCAGGAAAAGC-ACAGGG(A)30-3' NH2	CYP2D8wt(+) 1607b (A) 30-3'NH2
		-1603	
SEQ ID NO:65	2,	- GGGAGACCAGGGGGAGC-ATAGGGTTGGAGTGGGTGGT -3'	2D6(+)
SEQ ID NO:66	ς,	- GGGAGACCTTGTGGAGCGCCAGGGTTGGAGTGGGTGGC -3'	2D7(+)
SEQ ID NO:67	5,	- GGGAGACCAGGAAAAGC-ACAGGGTTGGAGTGGGCGGC -3'	2D8(+)

Pos/Neg Control probes- These probes were designed as true positive and negative control probes. They consist 5' Biotin. the same semi-random sequence, with the positive control probe having a . ထ of

CYP(+)ran(A)25-5'Biotin,3'NH2	CYP (+) ran (A) 25-3'NH2
5' Biotin- ATCATTCCAATCATCCATATCATC(A)25 -3' NH2	5'- ATCATTCCAATCATCCATATCATC(A)25 -3' NH2
SEQ ID NO:68	SEQ ID NO:69